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Research article

Optimization of feed composition for spray drying of probiotics

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Abstract
In this study, we are chosen *lactobacilli acidophilus* as probiotics and trying to optimize the feed composition to get the maximum protection to it during spray drying. Probiotics cell suspension keeps at 1%w/v on dry base and the three different protecting agents selected were microcrystalline cellulose, Lactose and skimmed milk. All were used at three different concentrations 5%w/v, 10%w/v and 15%w/v. Also MCC and skimmed milk were used in different combination. The results were analyzed for percent cell viability, percent recovery and moisture content as well as for powder properties. Maximum percent cell of 82% obtained with 15%w/v SM followed by 80% with 10%w/v SM. Lowest cell viability of 48% was obtained with 5%w /v MCC. Maximum recovery of 80% obtained with 5%w/v MCC and also gives the lowest moisture content of 3.3 percent. The spray dried samples were also analyzed for powder properties. Overall MCC and skimmed milk combination were found to be suitable for spray drying of probiotics.

Key words: spray drying, probiotics, *lactobacilli acidophilus.*

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1. Introduction

In 1907 the Russian scientist Elie Metchnikoff was the first to mention the beneficial effects of bacteria in his book "The prolongation of life" and presented the concept of "replacement of harmful microbes by useful microbes" [1-2] In 1906, Henry Tissier claimed that *bifidobacteria* are the dominant species in the microflora of breast-fed infants and recommended the administration of *bifidobacteria* to out-compete the putrefactive bacteria in infants suffering diarrhea. The term "probiotic" (Greek: pro life) was introduced by Lilly and Stillwell (1965) and after several re-definitions, the Joint Food and Agricultural Organization and World Health Organization [3] working group defined the term "probiotic" in 2001 as "live microorganisms that when administered in adequate amounts confer a health benefit on the host".
Probiotics research is now gaining importance as the probiotics not only considered as food additives or supplement but it is used as medicinal

agent. Viability in storage form is the major problem with probiotics. Probiotic preparations vary in the way in which they are presented; they may be in the form of powder, tablets, pastes or sprays with different excipients to maintain the preparation in the required condition. The type of preparation employed is determined by the way in which the probiotic is intended to be used. Preservation of frozen or freeze-dried biomolecules and cells is a complex topic, which affects a number of pharmaceutical, biotechnology, and food industries [4-5]. Because of its broad applicability, this is a highly active research area. Microscopic studies indicate that death after freezing and thawing is correlated well with membrane damage (i.e., rapture & leakage) [6-7].

However, there are many disadvantages associated with this approach; freeze drying is time-consuming and expensive, there are high transports and storage costs associated with frozen concentrated cultures, and in addition, freeze -thaw process results in loss of culture viability [8-9]. In comparison, spray drying, one of the predominant processing tools used in the dairy industry and in pharmaceuticals, can be used to produce large amounts of dairy ingredients relatively inexpensively; it has been estimated that the cost of spray drying is six times lower per kilogram of water removed than the cost of freeze-drying [10]. Spray dried powders can be transported at a low cost and can be stored in a stable form for prolonged periods. However, there are obvious challenges associated in using spray drying to produce viable cultures, including the requirement that the microorganisms survive the relatively high temperatures used [11]

2. Materials and Methods

Preformulation Studies:
Preformulation studies were performed for suitability of protecting agents with probiotics cells in suspension and solution form. Also these samples were analyzed for suitability of spray drying.

Strain identification and characterization:
Lyophilized sample of *lactobacilli acidophilus* were given by Sanzyme Ltd. Hyderabad, India as gift sample. The primary identification of the strain is based on gram staining and morphology. Molecular characterization by 16S rRNA partial gene sequencing is performed for strain identification.

Culturing, sub-culturing and isolation of Lactobacillus acidophilus:
Lactobacilli acidophilus lyophilized powder were suspended in distilled water and cultured on deMan, Rogosa and Sharpe (MRS- Hi-media, Mumbai) broth for 24 hrs at 37^{o}C. This freshly prepared culture of *Lactobacillus acidophilus* now were inoculated into MRS broth (1% v/v) and incubated at 37°C till the stationary phase was reached. After centrifugation at 8000 x g for 25 min at 4 °C, 1% (w/v) on wet weight basis was resuspended in distilled water.

Heat adaptation of feed culture:
To adapt change in temperature all the feed samples are exposed to 45^{o}C for 15 min prior to spray drying and maintained at 45^{o}C during entire spray drying process.

Optimizing parameters of feed composition for spray drying:
A spray dryer (Techno-search instruments, Mumbai) with a 0.7-mm two-

fluid nozzle was used. The solution was sprayed in a co-current flow with air as drying medium. The diameter of drying chamber was 9 inch and spray dryer attached with additional cyclone separator to minimize the loss.

Microcrystalline cellulose (MCC) as gift sample from Zim laboratories, Kalmeshwar, India, skimmed milk (SM) Himedia, Mumbai, and lactose, Himedia, Mumbai, were selected as protecting agent or bulking agent. These three agents are used at three different levels from 5%w/v to 15%w/v. the prepared probiotic cell suspension were used in such way final concentration of cell suspension was 1% (w/v). Optimization of feed solution process parameters and there rages given in table no. 1.

Table 1. The formulation code and ranges for optimization of feed composition

Probiotic	**Lactobacilli acidophilus cell suspension 1% (w/v)**								
Protecting agent	Skimmed milk			Lactose			MCC		
Formulation code	A1	A2	A3	A4	A5	A6	A7	A8	A9
Percent w/v	5	10	15	5	10	15	5	10	15
Fixed Spray dryer machine parameter	inlet air temperature: 140˚C, atomization pressure: 1.5 bar and feed rate: 4ml/min.								

Table 2. Effect of combination of protecting agent on spray drying

Probiotic	**Lactobacilli acidophilus cell suspension 1% (w/v)**					
Protecting agent	Skimmed milk: MCC					
Formulation code	B1	B2	B3	B4	B5	B6
Percent w/v	20:80	40:60	60:40	80:20	25:75	75:25
Fixed Spray dryer machine parameter	inlet air temperature: 140˚C, atomization pressure: 1.5 bar and feed rate: 4ml/min.					

To see the effect of combination protection agent the MCC and skimmed milk were used in different combination ratio as shown in table 2.

Evaluation of spray dried products:

Interaction studies: To evaluate the interactions of probiotics with MCC, skimmed milk and lactose, powder analyzed by Fourier transform Infrared spectroscopy (FT-IR) and differential scanning calorimetric (DSC). We have taken IR and DSC scans of all ingredients before spray drying, after spray drying and after one month storage.

Percent cell viability: Residual viability of spray dried samples was determined by the standard plate count method.

$$\text{Percent cell viability} = V/V0 \times 100$$

Where V0 and V represent the number of bacteria just before and after drying respectively [12-13]

Percent moisture content: Moisture content of spray dried powder which is defined as the ratio of dried water to initial powder weight, was determined by oven-drying at 102°. Determined by the measuring difference in weight before and after oven-drying

Percent recovery of the product: The spray dried powder recovery depends on the moisture contents and sticking of the products to cyclone separator and drying chamber.

Percent recovery= R/R0 × 100

Where R0 and R represent the gram of suspension before spray drying (on dry basis) and after drying respectively

Powder characteristics of the spray dried powder: spray dried powder were evaluated for Compressibility index and Hausner ratio.

Compressibility index (%) = 100× (tapped density-bulk density)/tapped density.

Hausner ratio= tapped density/bulk density.

3. Results and Discussion

Preformulation studies were carried out in which suitability of the protecting agent MCC, Skimmed milk and lactose in the suspension form analyzed with probiotic cell suspension. It was observed that all above selected protecting agent doesn't show any detrimental effect on probiotics. Primary identification morphology and staining was confirmed probiotic as *lactobacilli acidophilus* ATCC 43121. Culturing and sub culturing help to get freshly prepared bacterial strain. Heat adaptation was helpful for probiotic feed culture to adapt the drying temperature of spray dryer.

Optimizations of feed suspension parameter were observed for percent cell viability, moisture content and percent recovery as shown in table 3. The pre treatment was necessary to adapt the microorganism to sudden change in temperature and this pretreatment. Figure 1 indicates that the skimmed milk higher concentration gives the better protection but increasing concentration from 10%w/v to 15%w/v doesn't show any significant difference. MCC at lower concentration gives the better recovery of the product but shows the least protection. Higher concentration of MCC gives the comparable protection. Lactose gives the more percent cell viability than the MCC but shows the minimum product recovery and maximum moisture content. Minimum product recovery might be due to sticking of the lactose to the drying chamber and cyclone separator.

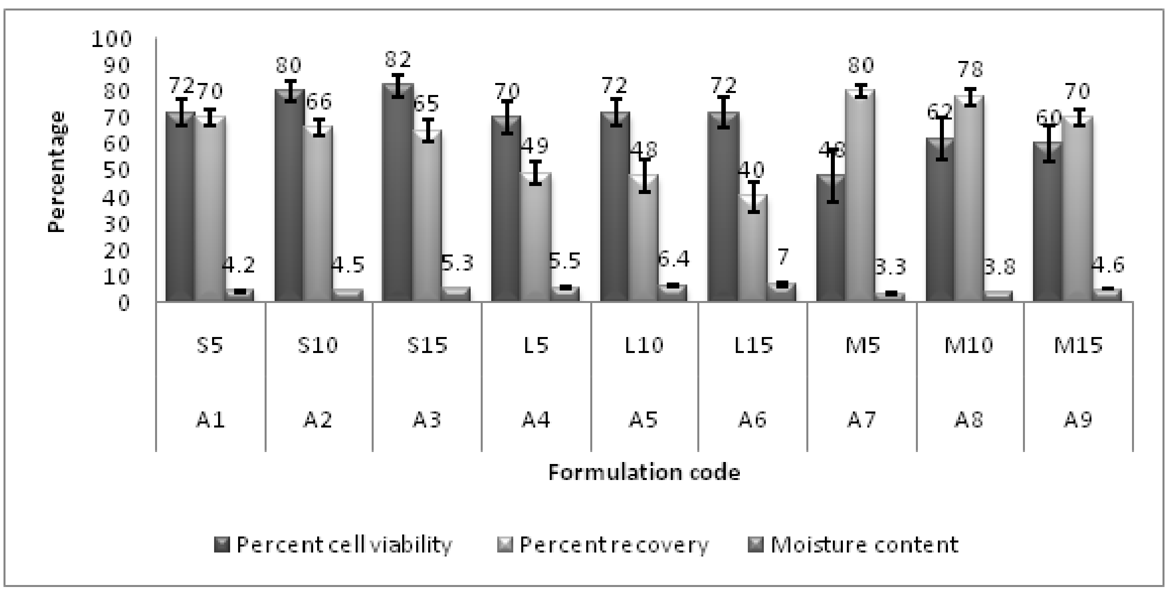


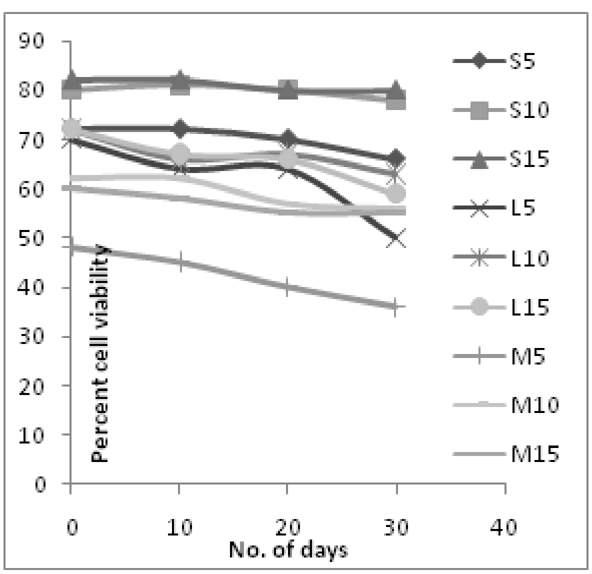
Figure 1. Feed composition and their response

Figure 2 indicates the cell viability during storage at room temperature and refrigerator temperature for 30 days which shows SM is gives the maximum protection even at room temperature. By taking powder properties in to consideration there was need for further optimization of feed composition by combination of skimmed milk and MCC (both at 10 percent w/v) and analyzed for same responses. The result of this is shown in table no.4. Figure 3 indicates that desired viability and powder characteristics was obtain with 40:60 ration of SM:MCC.

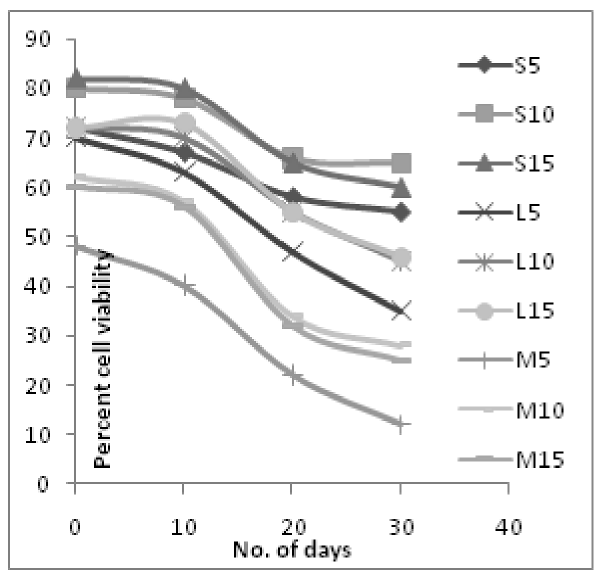
Table 3. Formulation code and ranges for optimization of feed composition with response

Probiotic	**Lactobacilli acidophilus cell suspension 1% (w/v)**								
Protecting agent	Skimmed milk			Lactose			MCC		
Formulation code	A1	A2	A3	A4	A5	A6	A7	A8	A9
Percent w/v	5	10	15	5	10	15	5	10	15
Percent cell viability	72±5	80±4	82±4	70±6	72±5	72±6	48±10	62±8	60±7
Moisture content	4.2±0.2	4.5±0.3	5.3±0.3	5.5±0.5	6.4±0.5	7.0±0.8	3.3±0.2	3.8±0.2	4.6±0.3
Percent recovery	70±3	66±3	65±4	49±4	48±6	40±6	80±2	78±3	70±3
Hausners ratio	1.26±0.04	1.26±0.03	1.30±0.04	1.22±0.02	1.24±0.03	1.25±0.04	1.22±0.03	1.19±0.03	1.17±0.02

Standard deviation is based on three readings.



(a)



(b)

Figure 2. Percent cell viability count of spray dried samples during one month storage a) at refrigerator condition and b) at Room temperature.

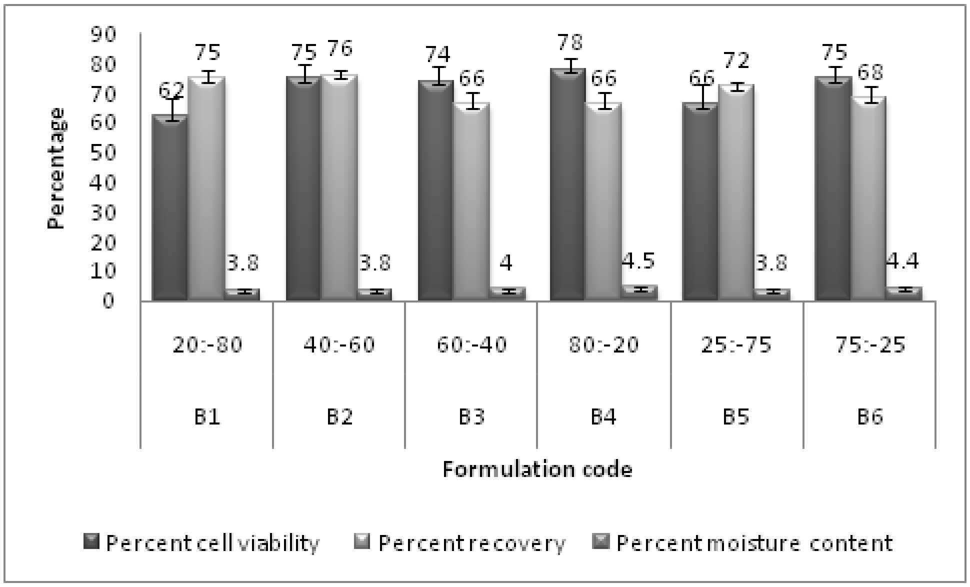


Figure 3. Effect of combination of protecting agent

Table 4. Formulation code and effect of combination of protecting agent

Formulation code	Ratio Skimmed milk: MCC	Percent cell viability	Percent recovery	Moisture content	Hausners ratio
B1	20:80	62±6	75±3	3.8±0.2	1.19±0.03
B2	40:60	75±5	76±2	3.8±0.3	1.20±0.02
B3	60:40	74±5	66±4	4±0.3	1.26±0.03
B4	80:20	78±4	66±4	4.5±0.5	1.27±0.04
B5	25:75	66±7	72±2	3.8 ±0.3	1.20±0.03
B6	75:25	75±4	68±4	4.4±0.4	1.28±0.03

Standard deviation is based on three readings.

Conclusion

Spray drying process was optimized with reference to feed composition Optimize drying were obtain by using combination of skimmed milk and MCC in the ratio 40:60. Both were used at 10%w/v as protecting agent with 1% *lactobacilli acidophilus* cell suspension. During entire study it was observed that the microorganism shows change in results even at same working conditions and this gives the big range of standard deviation. Once the feed composition optimize then optimization of machine parameters like inlet temperature, outlet temperature, feed rate, atomization pressure, dimension of drying chamber etc will becomes simple task.

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Conflict of interest: None

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